



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

January 30, 2020

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Chesterfield, MO 63017

Subject: PRIA and Non-PRIA (Pesticide Registration Improvement Act) Amendments to modify the terms of registration and product label for pink bollworm refuge and plantings in Puerto Rico
Product Name: MON 15985
EPA Registration Number: 524-522
Application Dates: July 12, 2019 and September 23, 2019
OPP Decision Numbers: 553325 and 556067

Dear Mr. Chi:

The amendments referred to above, submitted in connection with registration under Section 3(c)(5) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, are acceptable provided that you comply with the following terms and conditions.

1. Submit/cite all data required for registration of your product under FIFRA section 3(c)(5) when the Agency requires all registrants of similar products to submit such data.
2. The following information regarding test plots and seed production must be included in the grower guide for MON 15985 cotton and is a term of this registration:
 - a) No planting or sale for commercial planting of MON 15985 cotton is permitted south of Route 60 (near Tampa) in Florida.
 - b) No planting or sale for commercial planting of MON 15985 cotton is permitted in Hawaii, Puerto Rico, and the U.S. Virgin Islands.
3. The following restrictions apply to this product:
 - a) Test plots or breeding nurseries, regardless of the plot size, established in Hawaii must not be planted within 3 miles of *Gossypium tomentosum* and must be surrounded by 24 border rows of a suitable pollinator trap crop.

- b) Experimental plots and breeding nurseries of MON 15985 cotton are prohibited on the U.S. Virgin Islands, and
- c) Test plots or breeding nurseries, regardless of the plot size, may be established on the island of Puerto Rico without restriction if insecticide applications are used to effectively mitigate gene flow. Otherwise, test plots or breeding nurseries, regardless of the plot size, established on the island of Puerto Rico must not be planted within 3 miles of feral cotton plants and must be surrounded by 24 border rows of a suitable pollinator trap crop.

Upon approval by EPA, test plots and/or breeding nurseries in Hawaii, the U.S. Virgin Islands, and Puerto Rico may be established without restrictions if alternative measures, such as insecticide applications, are shown to effectively mitigate gene flow.

4. Insect Resistance Management Program Elements. The required IRM program for MON 15985 must have the following elements:

- a) Requirements for a non-*Bt* cotton refuge in conjunction with the planting of any acreage of MON 15985 in the states of Arizona, California, and New Mexico and in the following Texas counties: Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward, and Winkler. These requirements are not applicable if sampling shows that no pink bollworm are detected within these states or Texas counties;
- b) Requirements for Monsanto Company to prepare and require MON 15985 users to sign "grower agreements" which impose binding contractual obligations on the grower to comply with the IRM requirements;
- c) Requirements for Monsanto Company to develop, implement, and report to EPA on programs to educate growers about IRM requirements;
- d) Requirements for Monsanto Company to develop, implement, and report to EPA on programs to evaluate and promote growers' compliance with IRM requirements in the states of Arizona, California, and New Mexico and in the following Texas counties: Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward, and Winkler. These requirements are not applicable if sampling shows that no pink bollworm are detected within these states or Texas counties;
- e) Requirements for Monsanto Company to develop, implement, and report to EPA on programs to evaluate whether there are statistically significant and biologically relevant changes in susceptibility to Cry1Ac and Cry2Ab2 proteins in the target insects;
- f) Requirements for Monsanto Company to develop, and if triggered, to implement a "remedial action plan" which would contain measures Monsanto Company would take in the event that any insect resistance was detected as well as to report on activity under the plan to EPA;
- g) Requirements for annual reports on or before January 31st each year for compliance assurance. The tobacco budworm and cotton bollworm annual resistance monitoring reports must be submitted to EPA on or before June 30th each year and for pink bollworm, the annual resistance monitoring

report must be submitted to EPA on or before December 31st each year. See Annual Reports section below.

5. Insect Resistance Management Requirements

a) **Refuge Requirements for Pink Bollworm Resistance Management only in the states of Arizona, California, and New Mexico and in the following Texas counties: Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward, and Winkler.**

These refuge requirements are not applicable if no pink bollworm are found in monitoring traps in Arizona, California, New Mexico, and Texas (only the counties of Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward, and Winkler). In accordance with Section 5(e) of this registration notice, Monsanto Company is required to sample pink bollworm and monitor for resistance. Pink bollworm sampling may also be conducted by other Bt cotton product registrants, academic researchers, and/or the USDA. If pink bollworm are found in any of these sampling efforts, growers of MON 15985 must employ one of the following structured refuge options in the affected state or Texas counties in the subsequent growing season following detection, unless otherwise agreed by EPA:

i. External, Unsprayed Refuge

Ensure that at least 5 acres of non-*Bt* cotton (refuge cotton) is planted for every 95 acres of MON 15985. The size of the refuge must be at least 150 feet wide, but preferably 300 feet wide. This refuge may not be treated with sterile insects, pheromone, or any insecticide (except listed below) labeled for the control of tobacco budworm, cotton bollworm, or pink bollworm. At the pre-squaring cotton stage only, the refuge may be treated with any lepidopteran insecticide to control foliage feeding caterpillars. The refuge may be treated with acephate or methyl parathion at rates which will not control tobacco budworm or cotton bollworm (equal to or less than 0.5 lbs active ingredient per acre). The variety of cotton planted in the refuge must be comparable to MON 15985, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, termination, and management of other pests) similarly to MON 15985. Ensure that a non-*Bt* cotton refuge is maintained within at least 1/2 linear mile (preferably adjacent to or within 1/4 mile or closer) from the MON 15985 fields.

ii. External Sprayed Refuge

Ensure that at least 20 acres of non-*Bt* cotton are planted as a refuge for every 80 acres of MON 15985 (total of 100 acres). The variety of cotton planted in the refuge must be comparable to MON 15985, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, termination, and management of other pests) similarly to MON 15985. The non-*Bt* cotton may be treated with sterile insects, insecticides (excluding foliar *Btk* products), or pheromones labeled for control of the tobacco budworm, cotton bollworm, or pink bollworm. Ensure that a non-*Bt* cotton refuge is maintained within at least 1 linear mile (preferably within 1/2 mile or closer) from the MON 15985 fields.

iii. Embedded Refuge

Plant the refuge cotton as at least one single non-*Bt* cotton row for every six to ten rows of MON 15985 cotton. The refuge may be treated with sterile insects, any insecticide (excluding foliar *Btk* products), or pheromone labeled for the control of pink bollworm whenever the entire field is treated. The in-field refuge rows may not be treated independently of the surrounding MON 15985

field in which it is embedded. The refuge must be managed (fertilizer, weed control, etc.) identically to the MON 15985 cotton.

b) Natural Refuge Requirements for Tobacco Budworm and Cotton Bollworm Resistance Management only in the states of Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Missouri, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas (excluding the following counties: Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward, and Winkler), and Virginia.

1) The Monsanto Company must submit data to EPA by January 31st, 2022, and every five years thereafter, to support an EPA reassessment of the natural refuge and to confirm its effectiveness with tobacco budworm and cotton bollworm. The data must include: resistance monitoring data, cropping pattern analysis, and simulation modeling to reexamine levels of effective refuge in the states of Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Missouri, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia. Both cropping and land use patterns can change over time, which could impact the amount of natural refuge available to tobacco budworm and cotton bollworm relative to cotton. If based on this reassessment, EPA determines that additional tobacco budworm and/or cotton bollworm sampling, gossypol analysis, statistical analysis, and simulation modeling are needed to justify continuation of the natural refuge, Monsanto Company must submit these data within the EPA requested timeframe. If EPA's assessment concludes that the natural refuge is no longer scientifically supported, Monsanto Company has agreed and must submit an application to amend the registration to restore the structured refuge requirements previously required for tobacco budworm and cotton bollworm uses.

2) It is recommended that Monsanto Company develop a more complex, spatial model of resistance for MON 15985 that further considers the evolution of resistance "hotspots" (i.e. localized areas of resistance) and provide EPA with this information. Key issues like spatial structure, linkage disequilibrium, and differential movement of males and females have not yet been explored in detail for pyramided *Bt* proteins. Such models would be more desirable to examine the resistance evolution at the local level where natural refuge may be limited for one or more generations of tobacco budworm.

c) Grower Agreements.

While Monsanto Company will have flexibility to design its program to fit its own business practices, the registration is specifically conditioned on meeting the following requirements:

- 1) Persons purchasing MON 15985 must sign a grower agreement. The term "grower agreement" refers to any grower purchase contract, license agreement, or similar legal document.
- 2) The grower agreement and/or specific stewardship documents referenced in the grower agreement must clearly set forth the terms of the current IRM program. By signing the grower agreement, a grower must be contractually bound to comply with the requirements of the IRM program.
- 3) The Monsanto Company must continue to implement an approved system which is reasonably likely to assure that persons purchasing MON 15985 will affirm annually that they are contractually bound to comply with the requirements of the IRM program.

- 4) The Monsanto Company must continue to use an approved grower agreement. If Monsanto Company wishes to change any part of the grower agreement that would affect either the content of the IRM program or the legal enforceability of the provisions of the agreement relating to the IRM program, thirty days prior to implementing a proposed change, Monsanto Company must submit to EPA the text of such changes to ensure the agreement is consistent with the terms and conditions of this registration.
- 5) The Monsanto Company must continue an approved system which is reasonably likely to assure that persons purchasing MON 15985 sign grower agreement(s).
- 6) The Monsanto Company shall maintain records of all MON 15985 grower agreements for a period of three years from December 31st of the year in which the agreement was signed.
- 7) The Monsanto Company must maintain records detailing the number of units of the MON 15985 seed shipped and not returned and the number of such units that were sold to persons who have signed grower agreements. The Monsanto Company must submit the records to EPA within three months of the Agency's request.
- 8) The Monsanto Company must allow a review of the grower agreements and grower agreement records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that the names, personal information, and grower license number will be kept as confidential business information.

d) IRM Education and IRM Compliance Monitoring Programs.

The Monsanto Company must implement the following IRM education and compliance monitoring programs:

- 1) The Monsanto Company must design and implement a comprehensive, ongoing IRM education program designed to convey to MON 15985 users the importance of complying with the IRM program. The program shall include information encouraging MON 15985 users to pursue optional elements of the IRM program relating to refuge configuration and proximity to MON 15985 cotton fields. The education program shall involve the use of multiple media, e.g. face-to-face meetings, mailing written materials, and electronic communications such as by internet or television commercials. The program shall involve at least one written communication annually to each MON 15985 cotton grower separate from the grower agreement. Monsanto Company shall coordinate its education program with educational efforts of other organizations, such as the National Cotton Council and state extension programs.
- 2) Annually, Monsanto Company shall revise, and expand as necessary, its education program to take into account the information collected through the compliance survey required under paragraph 6 and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high. In addition, should the refuge requirements in section 5(a) be triggered, Monsanto Company shall ensure that the educational materials for that growing season include notification to MON 15985 growers of the requirements.

- 3) Within 90 days of request, MON 15985 shall provide a report to EPA summarizing the activities it carried out under its education program for the prior year and its plans for its education program during the current year.
- 4) The Monsanto Company shall continue to implement an ongoing, approved IRM compliance assurance program in the states of Arizona, California, and New Mexico and in the following Texas Counties: Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward, and Winkler. The program is designed to evaluate the extent to which growers are complying with the IRM program and that takes such actions as are reasonably needed to assure that growers who have not complied with the program either do so in the future or lose their access to MON 15985. Other required features of the program are described in paragraphs 5 - 12 below. These requirements are only applicable if refuge requirements have been triggered by the presence of pink bollworm (see section 5(a)).
- 5) The Monsanto Company shall establish and publicize a "phased compliance approach," i.e., a guidance document that indicates how Monsanto Company will address instances of non-compliance with the terms of the IRM program and general criteria for choosing among options for responding to any non-compliant growers. The options shall include withdrawal of the right to purchase MON 15985 for an individual grower or for all growers in a specific region. An individual grower found to be significantly out of compliance two years in a row would be denied sales of the product the next year.
- 6) The IRM compliance assurance program shall include an annual survey of a statistically representative sample of MON 15985 growers conducted by an independent third party. The survey shall measure the degree of compliance with the IRM program by growers in different regions of the country and consider the potential impact of non-response. The Monsanto Company shall provide a written summary of the results of the prior year's survey to EPA by January 31st of each year. The Monsanto Company shall confer with EPA on the design and content of the survey prior to its implementation.
- 7) Annually, Monsanto Company shall revise, and expand as necessary, its compliance assurance program to take into account the information collected through the compliance survey required under paragraph 6) and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high. The Monsanto Company will confer with the Agency prior to adopting any changes.
- 8) The Monsanto Company must conduct an annual on-farm assessment program. The Monsanto Company shall train its representatives who make on-farm visits with MON 15985 growers to perform assessments of compliance with IRM requirements. There is no minimum cotton acreage size for this program. Therefore, growers will be selected for this program from across all farm sizes. In the event that any of these visits results in the identification of a grower who is not in compliance with the IRM program, Monsanto Company shall take appropriate action, consistent with its "phased compliance approach," to promote compliance.
- 9) The Monsanto Company shall carry out a program for investigating "tips and complaints" that an individual grower or growers is/are not in compliance with the IRM program. Whenever an investigation results in the identification of a grower who is not in compliance with the IRM program, Monsanto Company shall take appropriate action, consistent with its "phased compliance approach."

10) If a grower, who purchases MON 15985 for planting, was specifically identified as not being in compliance during the previous year, Monsanto Company shall visit the grower and evaluate whether the grower is in compliance with the IRM program for the current year.

11) Annually by January 31, Monsanto Company shall provide a report to EPA summarizing the activities it carried out under its compliance assurance program for the prior year and its plans for its compliance assurance program during the current year. Included in that report will be the percent of growers using each refuge option (or combination of options) by region, the approximate number or percent of growers visited on farm by Monsanto Company, the number of tips investigated, the percent of growers who were not complying with the IRM requirements, and the follow-up actions taken.

12) The Monsanto Company must allow a review of the compliance records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that the names, personal information, and grower license number of the growers will be kept as confidential business information.

e) Insect Resistance Monitoring.

The Monsanto Company must conduct an annual resistance monitoring program for *Heliothis virescens* (tobacco budworm) *Helicoverpa zea* (cotton bollworm), and *Gossypiella pectinophora* (pink bollworm) for the Cry1Ac and Cry2Ab2 toxins expressed in MON 15985 as early as possible. Resistance monitoring programs must include: surveying insects for potential resistance and collection of information from growers about events that may indicate resistance. The Agency is imposing the following terms and conditions:

- 1) The Monsanto Company must utilize a revised MON 15985 (Cry1Ac and Cry2Ab2 toxins) resistance monitoring plan for *Heliothis virescens* (tobacco budworm) and *Helicoverpa zea* (cotton bollworm). The monitoring program must include increased sampling for tobacco budworm and cotton bollworm in the areas that have the greatest variability and potentially lowest levels of effective natural refuge. Sampling efforts should include all of the "worst-case" counties identified in the 2004 to 2006 analyses of natural refuge in the states of Texas, Tennessee, Mississippi, Louisiana, Arkansas, Alabama, Georgia, and North Carolina. BPPD believes that resistance monitoring for tobacco budworm and cotton bollworm resistance to Cry1Ac and Cry2Ab2 traits will have added importance with adoption of a natural refuge as a resistance management strategy.
- 2) The Monsanto Company must continue to develop and ensure the implementation of a plan for resistance monitoring for *Pectinophora gossypiella* (pink bollworm). The plan shall include provision for conducting annual studies to evaluate any potential change in susceptibility of pink bollworm populations to the Cry1Ac and Cry2Ab2 proteins using the discriminating dose, diagnostic dose, F2 screen, DNA markers, or other appropriate method, (specifically, the cadherin assay (B.E. Tabashnik, et al., J. Econ. Entomol. 99(5): 1525- 1530, 2006)). Collection sites must be focused in areas of high adoption, with the goal of including all states where pink bollworm is an economic pest.
- 3) The following testing scheme for survivors of the diagnostic or discriminating concentrations (or identified survivors of any resistance detection method) must be implemented: 1) Determine if the observed effect is heritable; 2) Determine if the increased tolerance can be observed in the field (i.e., survive on MON 15985 cotton plants); 3) Determine if the effect is due to resistance; 4) Determine the nature of resistance (dominant, recessive); 5) Determine the resistance allele frequency; 6) Determine,

in subsequent years, whether the resistance allele frequency is increasing; and 7) Determine the geographic extent of the resistance allele (or alleles) distribution. Should the resistance allele frequency be increasing and spreading, a specific remedial action plan should be designed to mitigate the extent of *Bt* resistance. See section f) ("Remedial Action Plans") below.

4) The Monsanto Company must also follow up on grower, extension specialist or consultant reports of less than expected results or control failures (such as increases in damaged squares or bolls) for the target lepidopteran pests (*Heliothis virescens* (TBW) and *Helicoverpa zea* (CBW), *Pectinophora gossypiella* (PBW)) as well as for cabbage looper, soybean looper, saltmarsh caterpillar, cotton leaf perforator and European com borer. The Monsanto Company will instruct its customers (growers and seed distributors) to contact them (e.g., via a toll-free customer service number) if incidents of unexpected levels of tobacco budworm, cotton bollworm, or pink bollworm damage occur. The Monsanto Company will investigate all damage reports. See Remedial Action Plans section below.

5) The Monsanto Company must provide to EPA for review and approval any revisions to the tobacco budworm, cotton bollworm, and pink bollworm resistance monitoring plans prior to their implementation.

6) A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by June 30th each year for tobacco budworm and cotton bollworm and by December 31st each year for pink bollworm for the duration of this registration.

f) Remedial Action Plans.

Specific remedial action plans are required for MON 15985 for the purpose of containing resistance and perhaps eliminating resistance if it develops. One remedial action plan is for the area where pink bollworm is the predominate pest and the other is for the area where tobacco budworm and cotton bollworm are the predominate pests.

1) Remedial (Mitigation) Action Plan for Tobacco Budworm and Cotton Bollworm (Attachment I)

If resistance involves the tobacco budworm (*Heliothis virescens*) and/or the cotton bollworm (*Helicoverpa zea*), Monsanto Company must implement the Remedial Action Plan approved by EPA. Monsanto Company must obtain approval from EPA before modifying the Remedial Action Plan for Cotton Bollworm and Tobacco Budworm.

2) Remedial Action Plan for Pink Bollworm (Attachment II)

If resistance involves the pink bollworm (*Pectinophora gossypiella*), Monsanto Company must implement the Arizona *Bt* Cotton Working Group's Remedial Action Plan. Monsanto Company must obtain approval from EPA before modifying the Arizona *Bt* Cotton Working Group's Remedial Action Strategy.

g) Annual Reports for Sales, Grower Education, Compliance Assurance, Grower Agreements, and Resistance Monitoring.

- Annually by January 31st Monsanto Company will provide to the EPA a report that contains the results of the compliance assurance program.

- The Monsanto Company will provide to EPA within 90 days of request:
 - Annual sales reported and summed by state (county level data available by request);
 - A report summarizing the number of units of *Bt* cotton seeds shipped or sold and not returned and the number of such units that were sold to persons who have signed grower agreements;
 - A report summarizing any substantive changes to the grower education program completed the previous year.

- A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by June 30th each year for tobacco budworm and cotton bollworm and by December 31st each year for pink bollworm for the duration of this registration.

Your release for shipment of this product constitutes acceptance of these terms. If these terms are not complied with, this registration will be subject to cancellation in accordance with FIFRA section 6.

A revised label reflecting the changes in terms and conditions above is attached to this amendment approval letter.

If you have any questions, please contact Michael Glikes of my team by phone at (703) 305-6231 or via email at glikes.michael@epa.gov.

Sincerely,



Alan Reynolds, Team Leader
Emerging Technologies Branch
Biopesticides and Pollution
Prevention Division (7511P)
Office of Pesticide Programs

Attachments

**A Plan for Monitoring and Mitigating Resistance to Bollgard II®
Cotton in Heliothine Pests
November 12, 2004**

I. RATIONALE

Bollgard II® cotton is an important pest management tool for U.S. cotton farmers. Since the Bollgard® cotton introduction in 1996, this technology has provided economical and effective control of two key heliothine pests, the tobacco budworm (TBW), *Heliothis virescens*, and the cotton bollworm (CBW), *Helicoverpa zea*. The deployment of Bollgard cotton has ended the cotton farmers' near total reliance on chemical insecticides for the management of heliothine insects. However, the evolution of resistance in heliothine pests to the Cry1Ac protein expressed in Bollgard cotton cultivars is a potential threat to the sustainability of Bollgard cotton. Bollgard II cotton was developed as a two-gene product expressing both Cry1Ac and Cry2Ab2 proteins in an attempt to mitigate potential resistance evolution. As a condition of registration of Bollgard II cotton (EPA Reg. No. 524-522), the U.S. EPA required Monsanto Company to develop and implement a program to monitor for insect resistance to the Cry1Ac protein expressed in both Bollgard cotton and Bollgard II cotton as well as the Cry2Ab2 protein expressed only in Bollgard II cotton, and direct mitigation actions against resistance if it were to occur to the Bollgard II cotton product. This document describes a Remedial Action Plan for Bollgard II cotton.

II. COMPONENTS AND ORGANIZATION

The registrant is responsible for organizing, deploying, and financially supporting the Bollgard II cotton resistance monitoring and mitigation program for TBW and CBW.

Agricultural consultants, state and federal entomologists, growers, dealers' field personnel, and others will be enlisted, as volunteers and contractors, to assist in surveying, detection, verification, and other components of the monitoring program. To the extent possible, the registrant will educate and encourage persons working in cotton pest management to be conscious of and alert to resistance evolution events. When appropriate, the registrant will hire qualified individuals to execute specific protocols for resistance monitoring, such as for the testing of suspected resistant insects, and enlist necessary expertise, assistance, supplies, etc., for the conduct of a successful program.

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III. MONITORING APPROACHES

Monitoring provides the capability of detecting the presence of rare resistance alleles in field populations, even though product failure may not be occurring. If the monitoring programs demonstrate the presence of field resistance in a population, then steps will be taken to characterize the resistance.

A. Susceptibility Monitoring

Monitoring for resistance evolution to Bollgard II cotton in TBW and CBW will also include the ongoing USDA/ARS project at Stoneville, MS. The goal of this program is to detect early changes in the frequency of Cry1Ac and/or Cry2Ab resistance alleles in TBW and CBW. Data from the program may have early detection benefits and may provide direction for the in-field monitoring activity.

Monsanto also will continue to work with academic experts to evaluate the suitability of alternative monitoring and screening methods as discoveries are made on the nature of Cry1Ac and Cry2Ab2 insect resistance.

B. Integrated Pest Management (IPM)-Based Monitoring

Monitoring for the early detection of resistance to Bollgard II cotton poses several significant challenges. The program will require insect sampling each season when TBW and CBW are infesting cotton and must be conducted across most of the U.S. cotton belt. One reasonable and cost effective approach is IPM-based monitoring. A majority of cotton fields in the U.S. are normally scouted for heliothine pest insects on an annual basis. IPM consultants (and their employed scouts), cotton growers, and/or commercial agricultural dealers selling crop services and products routinely monitor insect pests on a weekly or more frequent basis each season. The IPM-based monitoring strategy integrates resistance monitoring with regular cotton scouting, other pest management observations, and those also conducted on Bollgard cotton for TBW and CBW. The approach potentially taps the efforts of hundreds of pest management consultants and commercial representatives and thousands of cotton scouts.

IPM consultants, growers and/or commercial representatives will be requested to notify the registrant of unusual TBW and/or CBW survival in Bollgard II cotton. The registrant will work with IPM consultants, growers, and commercial representatives and communicate the need to implement IPM-based monitoring for resistance. The communication may include seminars, brochures, videos, presentations, and other effective techniques. The IPM-based monitoring will be effectively marketed and may include promotions and other forms of encouragement. A toll-free number and other opportunities will be used to facilitate communication between the field team conducting the monitoring and the registrant. The registrant will support this program with the supplies needed (e.g., protocol, insect collection instructions, supplies, etc.).

TBW and/or CBW larvae with unusual survival will be collected from Bollgard II cotton fields and held on non-B.t. plant tissue or placed on appropriate diet for identification and testing purposes. The registrant will coordinate this effort with state and ARS entomologists to provide directions for collecting/holding larvae and to make insect diet available in a timely manner.

IV. DEFINITIONS

A. Resistance Event, Putative-One or more of the following will define a putative resistance event after confirmation of the plant genotype:

1. Susceptibility Monitoring:

Significantly elevated survival of TBW and/or CBW larvae in a standardized laboratory diagnostic dose assay at the 95% confidence level compared to baseline data for susceptible populations.

2. IPM-Based Monitoring:

- a. Survival of TBW to second or later instar with at least two larvae per 100 flowers, squares, and bolls (proportional to the plants fruiting profile) with: 1] normal Cry1Ac and Cry2Ab2 protein titer in Bollgard II cotton plants, and 2] Bollgard II cotton plant populations of $\geq 98\%$ purity, plus verification of statistically significant survival at the 95% confidence level as compared to appropriate baseline data for TBW.
- b. Survival of CBW to third or greater instar with ≥ 15 larvae per 100 flowers, squares, and bolls (proportional to the plants fruiting profile) with: 1] confirmation that the CBW infestation was typical (an abnormally high CBW infestation may necessitate a higher threshold), 2] normal Cry1Ac and Cry2Ab2 protein titer in Bollgard II cotton plants, and 3] a Bollgard II cotton plant population of $\geq 98\%$ purity, plus verification of statistically significant survival at the 95% confidence level as compared to appropriate baseline data for CBW.

B. Resistance Event, Confirmation

Resistance to Bollgard II cotton will be confirmed by verification of statistically significant survival at the 95% confidence level as compared to appropriate baseline data. Confirmation of results in the same testing laboratory or another laboratory can be considered as appropriate. Baseline ranges of Cry1Ac LC₅₀ values for control of TBW and/or CBW have been reported in the literature (Stone and Sims, 1993; Luttrell et al., 1999) and Cry2Ab2 LC₅₀ values for control of TBW and/or CBW are currently being established from 2002 and 2003 data.

V. MITIGATION ACTIONS

A. *Resistance Event, Putative*

Surveys, plant tissue collections, and/or in-field analysis will be conducted as needed to determine the genotype purity of the plant population and expressed titer of Cry1Ac and Cry2Ab2 in infested Bollgard II cotton plants.

The registrant will notify the appropriate state CES entomologist(s) working with cotton after confirmation of plant genotype and request his/her cooperation to further clarify the putative resistance event. Fields within the vicinity of a putative resistance field(s) (e.g., within 1 mile) will be thoroughly sampled for further evidence of resistance. If substantiating data are gathered, the sampling will be expanded to obtain a preliminary estimate of the extent of increased TBW and/or CBW survival. To this end, interviews with consultants, extension agents, and growers will be conducted.

Collections of TBW and/or CBW will be made for establishment of laboratory colonies. Moths and larvae will be collected from the infested field(s) and from other Bollgard II cotton fields identified in the survey. Progeny from the colony(s) will be subjected to standard bioassay testing.

After confirmation of the Bollgard II cotton genotype in the affected field, and depending on the timing, the extent and the nature of the resistance, the registrant may instruct growers to use alternative control measures to control the pest suspected of resistance in the Bollgard II cotton region with the detection. In addition, the registrant may also instruct growers to destroy crop residues in the affected region (i.e., within one month) with a technique appropriate for local production practices to minimize the possibility of resistant insects over-wintering and contributing to the next season's pest population.

B. *Resistance Event, Confirmed*

The registrant assumes responsibility for the implementation of resistance mitigation actions undertaken in response to the occurrence of resistance during the growing season. In cases of *confirmed* resistance, the following strategy for Bollgard II cotton varieties will be implemented:

The registrant will report all instances of confirmed pest resistance, as defined above, to the Agency within 30 days. Upon identification of a confirmed instance of resistance, registrants will take the following mitigation measures immediately:

1. Notify customers and extension agents in the affected area;

2. Instruct that customers and extension agents in the affected area use additional control measures to reduce or control the local target pest population;
3. If circumstances deem it appropriate (the pest, the extent of resistance, the timing of the resistance and the nature of the resistance), require that customers and extension agents in the affected area incorporate crop residues into the soil following harvest, to minimize the possibility of over-wintering insects; and
4. If circumstances deem it appropriate (the pest, the extent of resistance, the timing of the resistance and the nature of the resistance), stop sale and distribution of Bollgard II cotton immediately in the remedial action zone (may be a single county or multiple counties) where the resistance has been shown until an effective local mitigation plan approved by EPA has been implemented.

Within 90 days of a confirmed instance of pest resistance, as defined above, the registrant will:

1. Notify the Agency of the immediate mitigation measures that were implemented;
2. Submit to the Agency a proposed long-term resistance management action plan for the affected area;
3. Work closely with the Agency in ensuring that an appropriate long-term resistance management action plan for the affected area is implemented;
4. Implement an action plan that is approved by EPA, consisting of some or all of the following elements as appropriate:
 - a. Inform customers and extension agents in the affected area of pest resistance;
 - b. Increase monitoring in the affected area and ensure that local target pest populations are sampled on an annual basis;
 - c. Recommend additional measures to reduce or control target pest populations in the affected area;
 - d. Implement intensified local IRM measures in the affected area based on the latest research results; and
 - e. Coordinate the implementation of the remedial action strategy by the Agency with other stakeholders.

For mitigation of resistance in the growing season(s) following the confirmed resistance incident(s), the registrant will recommend appropriate measures based upon considering

the pest, the extent of the resistance, and the nature of the resistance identified. The registrant will recommend use of some or all of the following procedures:

1. Notification of all relevant personnel (e.g., growers, consultants, extension agents, seed distributors, processors, university cooperators, and state/federal authorities) in the affected region of the resistance situation;
2. Intensified monitoring and surveillance in the affected region(s) and definition of the boundaries of the affected region. These studies could also include assays to determine the potential for cross-resistance in the resistant population;
3. The development and use of alternative resistance management strategies for controlling the resistant pest(s) on cotton in the affected region;
4. Where sales have been suspended, maintenance of the suspension of all Bollgard II cotton products and similar products with one or more of the same B.t. proteins in the affected region, which would remain in place until susceptibility has been determined to have returned to acceptable levels; and
5. If EPA agrees that an effective local resistance management plan has been implemented which mitigates resistance, the registrant can resume sales in the affected county or counties.

VI. REFERENCES

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Cooperative Extension



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--Reviewed 3 June, 2002, by the AZ Bt Cotton Working Group--

A Remedial Action Plan for Responding to Pink Bollworm Resistance to Bt Cotton in Arizona

*Formulated by the Arizona Bt Cotton Working Group
T.J. Dennehy, Chair*

I. Definitions

Definition #1. Putative Resistance Event--A Cautionary Alert

A putative resistance event consists of any field of Bt cotton in which collections of 100 bolls yield $\geq 3\%$ large larvae (≥ 3 rd instar), pupae or PBW exit holes in bolls. This is a cautionary alert and must not be construed to be a verified resistance event until: 1) the plants from which collections were made are confirmed to produce Bt toxin and, 2) bioassays are completed that confirm the reduced susceptibility of the pink bollworm surviving on Bt cotton.

Definition #2. A Verified Resistance Event.

A putative resistance event becomes verified if three conditions are met:

- 1) A sample of 1000 bolls yields $\geq 3\%$ containing large larvae (≥ 3 rd instar), pupae, or PBW exit holes.
- 2) An ELISA test for Bt toxin yields a positive response for Bt toxin in a sample of 25 young bolls collected from plants on which PBW larvae were found in the cotton field of interest.
- 3) Standardized laboratory bioassays demonstrate that the PBW population of interest is significantly less susceptible to Cry1A(c) toxin than were baseline populations in 1997 (Simmons et al. 1998 and unpublished).

II. Remedial Action

- 1) Putative Resistance Event: Year of First Detection.

Within one week of confirming that a Bt field has $\geq 3\%$ of bolls containing large larvae (≥ 3 rd instar), pupae, or PBW exit holes, alternative PBW controls should be implemented in that field. Measures should include one or more of the following:

- Adulticide treatments if crop is in active growing state, followed by additional insecticide applications (2) on a 3-day schedule, or based on adult emergence as predicted by phenological models.

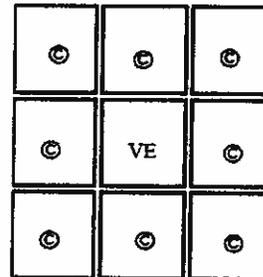
U-EXTENSION
A-ARTHROPOD
RESISTANCE
MANAGEMENT
LABORATORY

- If crop is senescent, consider chemical termination to reduce squares and bolls less than 10 days old, accelerate harvest, and destroy crop residue by shredding of stalks followed by discing, and deep plowing (6" burial).
- If crop is defoliated, accelerate harvest and destruction of crop residue to further limit survival of resistant pink bollworm. Destroy crop residue as indicated above.

2) Verified Resistance Event: Year of First Detection.

A. If resistance is verified in time to permit it, we strongly recommend that measures be taken to reduce the numbers of resistant pink bollworm that survive to the next season. These could include: adulticide treatments, early termination, and early plowdown, consisting of shredding of stalks followed by discing, and deep plowing (6" burial). Winter irrigation is also recommended to reduce survivorship of overwintering larvae.

B. Bt fields in the immediate vicinity of a verified resistance event should be examined to detect unusual survivorship of PBW. Results should be used to delimit the size of the affected area and to define the 'Bt remedial action zone.' We suggest sampling 300 bolls from all Bt fields located within the 8 sections of land (designated by ☉ in the adjacent figure) that surround the section of land on which the verified event (VE) occurred. Bt cotton fields containing $\geq 3\%$ bolls infested with PBW should be considered affected by resistance for the purpose of delimiting the remedial action zone.



- C. The 'Bt remedial action zone' should be delineated using GPS mapping technology currently in use at the ACRPC. This will ensure accurate records of locations of verified resistance. The remedial action zone should include all sections of land falling within 6 miles of the perimeter of the section(s) of land in which verified resistance events occurred (see figure below).
- D. At such time as fields with verified resistance are detected in >3 different townships within a particular cotton growing region, the entire region may be designated as a Bt resistance remedial action zone.

3) Verified Resistance Event: Next Year's Actions.

A. If verified resistance occurred in only Bollgard, then only Bollgard II, or non-Bt cotton should be planted in the remedial action zone in the year(s) immediately following verification of resistance. If verified resistance occurred in Bollgard and/or Bollgard II, then only non-Bt cotton should be planted in the remedial action zone in the year(s) immediately following verification of resistance. These measures should be maintained until such time as bioassays of PBW from the remedial action zone demonstrate that the frequency of resistant individuals has declined to acceptable levels. What will constitute levels of resistance acceptable for allowing resumption of use of Bt cotton will be determined on an *ad hoc* basis by our Working Group, based on research experience that members have obtained from studies of pink bollworm resistance to Cry1Ac.

The ecological fitness of PBW resistant to Cry1Ac is not known at this time and the dynamics of resistance in the field will likely be influenced by factors including overwintering survival of resistant

larvae, intensity of resistance to Cry1Ac, and growth and survival of resistant PBW on Bt and non-Bt plants. Therefore, new information derived from field and laboratory studies currently underway will be pivotal for determining the frequency of resistance (to Cry1Ac or to mixtures of Cry1Ac and Cry2Ab2) at which use of Bt cotton could reasonably be resumed within an area previously designated as a Bt remedial action zone.

- B. It is assumed that published University recommendations for monitoring and chemical control of pink bollworm will be followed within remedial action zones in order to limit survival of resistant pink bollworm. Additionally, timely crop termination (no top-crop) and early and thorough crop destruction, as detailed above, is strongly encouraged. Releases of sterile pink bollworm should also be considered.
- C. The recommendations of our working group regarding 1) Bt refuge management and 2) remedial action for responding to PBW resistance in Arizona should be re-evaluated annually and modified to account for new findings. Educational programs and regulatory measures should be devised to promote a high level of producer compliance with recommendations.

III. Organizational Roles

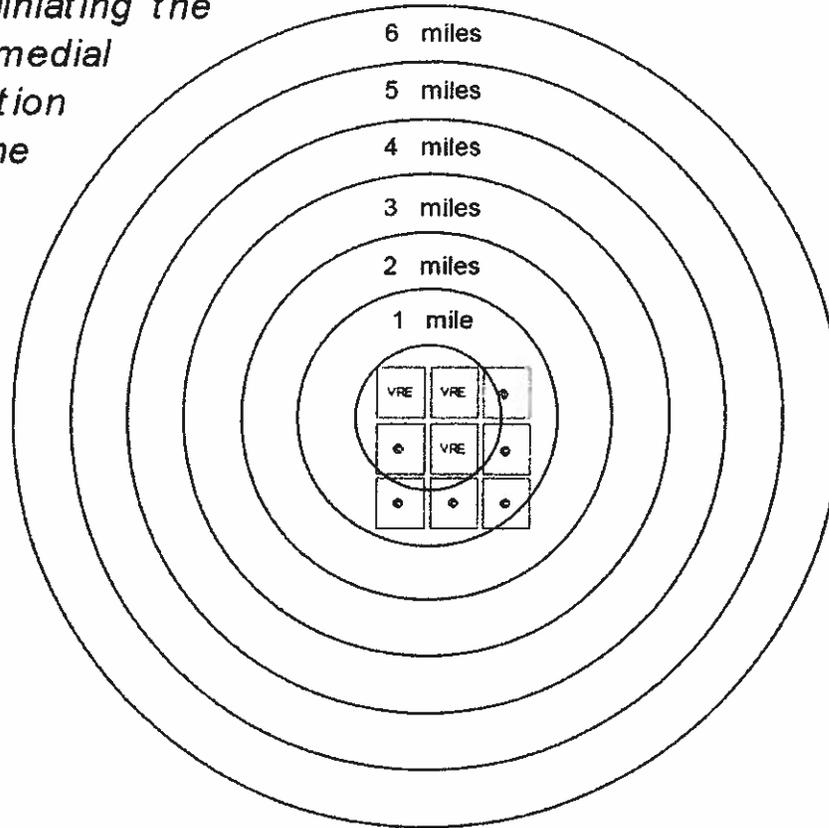
- 1) The Arizona Department of Agriculture should serve a central role in implementing this plan, compiling statistics on use of Bt cotton, and promoting compliance with remedial action.
- 2) Consideration should be given on a case-by-case basis for making funds available to compensate producers for costs associated with implementing the remedial action measures recommended herein.
- 3) A sampling team comprising personnel from relevant organizations (ACRPC, UA, USDA) will be formed. This team will be ready in August of every year to conduct the sampling required to delineate resistance problems (as detailed above). Similarly, facilities and personnel at EARML will be prepared to conduct bioassays or molecular tests of up to 40 different populations of PBW per season. Funding for these efforts must be sustained.
- 4) Monsanto should agree to suspend Bt cotton sales in remedial action zones until such time as either the frequency of resistant individuals is shown to have declined to levels deemed acceptable by our Working Group, or new Bt products free of cross-resistance are introduced, and the Arizona Bt Cotton Working Group has concluded that a modified resistance management strategy has been adopted that will adequately reduce the rate of development of further resistance to Bt cotton products.

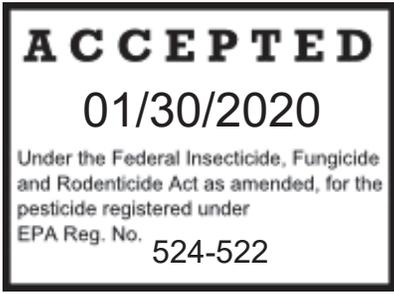
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- a. Simons, A.L., T.J. Dennehy, B.E. Tabashnik, L. Antilla, A. Bartlett, D. Gouge and R. Staten. 1998. Evaluation of B.t. cotton deployment strategies and efficacy against pink bollworm in Arizona. Proc. 1998 Beltwide Cotton Conferences. 1025-1030.
- b. Liu, Y.-B., Tabashnik, B.E., Dennehy, T.J., Patin, A.L., and Bartlett, A.C. 1999. Development time and resistance to Bt crops. Nature 400:519.
- c. Patin, A.L., Dennehy, T.J., Sims, M.A., Tabashnik, B.E., Liu, Y.B., Antilla, L., Gouge, D., Hemeberry, T.J., and R. Staten. 1999. Status of pink bollworm susceptibility to Bt in Arizona. Proc. Beltwide Cotton Conferences. National Cotton Council. Pp. 991-996.

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*Deliniating the
Remedial
Action
Zone*





Plant-Incorporated Protectant Label

MON 15985

(OECD Unique Identifier: MON-15985-7)

Active Ingredients:

Bacillus thuringiensis Cry2Ab2 protein and the genetic material necessary for its production
[PV-GHBK11] in event MON 15985 cotton < 0.009%*

Bacillus thuringiensis CryIAc protein and the genetic material necessary for its production
[PV-GHBK04] in event MON 15985 cotton < 0.00052%*

Other Ingredients:

Substance produced by the marker genes and the genetic material necessary for their production
[PV-GHBK04 and PV-GHBK11] in event MON 15985 cotton < 0.0304%*

*Percentage (wt/wt) on a dry weight basis.

KEEP OUT OF REACH OF CHILDREN

CAUTION

EPA Registration Number 524-522
EPA Establishment Number 524-M0-002

Monsanto Company
800 North Lindbergh Blvd.
St. Louis, Missouri 63167

NET CONTENTS _____

DIRECTIONS FOR USE

It is a violation of federal law to use this product in a manner inconsistent with its labeling.

MON 15985 has been transformed to express the *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) delta endotoxin CryIAc and Cry2Ab2 proteins for the control or suppression of the following lepidopteran cotton insect pests:

Tobacco Budworm	<i>Heliothis virescens</i>
Pink Bollworm	<i>Pectinophora gossypiella</i>
Cotton Bollworm	<i>Helicoverpa zea</i>
Cabbage Looper	<i>Trichoplusia ni</i>
Saltmarsh Caterpillar	<i>Estigmene acrea</i>
Cotton Leaf Perforator	<i>Bucculatrix thurbeiella</i>
Soybean Looper	<i>Pseudoplusia includes</i>
Beet Armyworm	<i>Spodoptera exigua</i>
Fall Armyworm	<i>Spodoptera frugiperda</i>
Yellowstriped Armyworm	<i>Spodoptera ornithogolli</i>
European Corn Borer	<i>Ostrinia nubilalis</i>

Transformed cotton must be accompanied by the Grower Guide, which contains the following:

1. The *B.t.k.* delta endotoxin proteins expressed in this product control or suppress the above listed lepidopteran cotton insect pests.
2. Routine applications of insecticides to control these insects are usually unnecessary when cotton containing the *B.t.k.* delta endotoxin proteins is planted.
3. Instruction for growers to read the product Grower Guide prior to planting for information on planting, production and insect-resistance management.
4. This product must not be planted nor sold for commercial planting in Hawaii, Puerto Rico, U.S. Virgin Islands, and south of Route 60 (near Tampa) in Florida.

The following restriction apply to this product:

- a) Test plots or breeding nurseries, regardless of the plot size, established in Hawaii must not be planted within three miles of *Gossypium tomentosum* and must be surrounded by 24 border rows of a suitable pollinator trap crop.
- b) Experimental plots and breeding nurseries of this product are prohibited on the U.S. Virgin Islands, and
- c) Test plots or breeding nurseries, regardless of plot size, may be established on the island of Puerto Rico without restriction if insecticide applications are used to effectively mitigate gene flow. Otherwise, test plots or breeding nurseries, regardless of plot size, established on the island of Puerto Rico must not be planted within three miles of feral cotton and must be surrounded by 24 border rows of a suitable pollinator trap crop.

Refuge Requirements for Pink Bollworm Resistance Management only in the states of Arizona, California, and New Mexico and in the following Texas counties: Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward, and Winkler.

These refuge requirements are not applicable if no pink bollworm are found in monitoring traps in Arizona, California, New Mexico, and Texas (only the counties of Brewster, Crane, Crockett, Culberson, El Paso, Hudspeth, Jeff Davis, Loving, Pecos, Presidio, Reeves, Terrell, Val Verde, Ward, and Winkler). In accordance with the terms of this registration, Monsanto Company is required to sample pink bollworm and monitor for resistance. Pink bollworm sampling may be conducted by other Bt cotton product registrants, academic researchers, and/or the USDA. If pink bollworm are found in any of these sampling efforts, growers of this product must employ one of the following structured refuge options in the affected state or Texas counties in the subsequent growing season following detection, unless otherwise agreed by EPA:

1) External, Unsprayed Refuge

Ensure that at least 5 acres of non-*Bt* cotton (refuge cotton) is planted for every 95 acres of this product. The size of the refuge must be at least 150 feet wide, but preferably 300 feet wide. This refuge may not be treated with sterile insects, pheromone, or any insecticide (except listed below) labeled for the control of tobacco budworm, cotton bollworm, or pink bollworm. At the pre-squaring cotton stage only, the refuge may be treated with any lepidopteran insecticide to control foliage feeding caterpillars. The refuge may be treated with acephate or methyl parathion at rates which will not control tobacco budworm or cotton bollworm (equal to or less than 0.5 lbs active ingredient per acre). The variety of cotton planted in the refuge must be comparable to this product, especially in the maturity date, and the refuge must be managed (*e.g.*, planting time, use of fertilizer, weed control, irrigation, termination, and management of other pests) similarly to this product. Ensure that a non-*Bt* cotton refuge is maintained within at least 1/2 linear mile (preferably adjacent to or within 1/4 mile or closer) from the MON 15985 cotton fields.

2) External Sprayed Refuge

Ensure that at least 20 acres of non-*Bt* cotton are planted as a refuge for every 80 acres of this product (total of 100 acres). The variety of cotton planted in the refuge must be comparable to this product, especially in the maturity date, and the refuge must be managed (*e.g.*, planting time, use of fertilizer, weed control, irrigation, termination, and management of other pests) similarly to this product. The non-*Bt* cotton may be treated with sterile insects, insecticides (excluding foliar *Btk* products), or pheromones labeled for control of the tobacco budworm, cotton bollworm, or pink bollworm. Ensure that a non-*Bt* cotton refuge is maintained within at least 1 linear mile (preferably within 1/2 mile or closer) from the MON 15985 cotton fields.

3) Embedded Refuge

Plant the refuge cotton as at least one single non-*Bt* cotton row for every six to ten rows of this product. The refuge may be treated with sterile insects, any insecticide (excluding foliar *Btk* products), or pheromone labeled for the control of pink bollworm whenever the entire field is treated. The in-field refuge rows may not be treated independently of the surrounding *Bt* field in which it is embedded. The refuge must be managed (fertilizer, weed control, etc.) identically to the MON 15985 cotton.